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A New Anorexigen Assay: Stress-Induced Hyperphagia in Rats

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WALLACH, M. B., M. DAWBER, M. MCMAHON AND C. ROGERS. *A new anorexigen assay: stress-induced hyperphagia in rats.* PHARMAC. BIOCHEM. BEHAV. 6(5) 529–531, 1977. – Humans often respond to stress by overeating. A rat model of hyperphagia induced by a stressful tail-pinch has been described. Consumption of sweetened condensed milk by naive rats was measured during the stress. This milk drinking was prevented by pretreatment with d-amphetamine, chlorphentermine, diethylpropion, fenfluramine, methamphetamine, morphine, chlorpromazine and haloperidol. The benzodiazepines chlordiazepoxide and diazepam increased the hyperphagia. The relevance of dopaminergic agonistic and antagonist activities to anorexigenic activity are questioned.

Anorexigen assay Hyperphagia d-Amphetamine Chlorphenteramine Diethylpropion Fenfluramine Methamphetamine Morphine Chlorpromazine Haloperidol Chlordiazepoxide Diazepam

THE INDUCTION of hyperphagia in rats during a mildly stressful tail-pinch has been described [1,4]. This syndrome is characterized by hyperphagia or polydipsia upon presentation of food or drink during a mildly stressful tail-pinch. This syndrome can be antagonized by neuroleptic agents which also are dopamine antagonists [2]. This is curious because known anorexigenic agents are often dopaminergic stimulants [6].

It has often been observed that humans, exposed to some stress, e.g., stopping smoking or final examinations in school, will often increase their food consumption. Because of this possible parallel to the human situation, this model of tail-pinch-induced hyperphagia has been explored as a possible screen for anorexigenic compounds.

METHOD

Groups of seven to ten naive male Sprague-Dawley rats (Hilltop) weighing 120–180 g were utilized for these experiments. Each rat was used only once. The rats were maintained with ad lib access to standard rat chow and water until the time of the experiment. The rats were treated with either the vehicle or drug prior to testing.

For the test, the rat was placed in a cylindrical plastic restraining tube 19 cm long with an inside diameter of 4.7 cm. Each tube had 36 air holes in the side and adjustable end-plates allowing the tail to exit from one end, and the insertion of a metal drinking tube into the other end. The restraint tubes were placed on end with the tail exposed.

The drinking tube was filled with two parts sweetened condensed milk (Borden's Eagle Brand) and one part water. The reservoir for the drinking tube consisted of a 25 ml pipette which had been modified with a ground glass stopper at the top. The flared bottom of the pipette was connected to the metal drinking tube with Tygon tubing. The metal drinking tube was inserted into the top of the restraint cylinder and a clothespin was placed about 3 cm from the tip of each tail for 15 min. The rats, unaccustomed to the milk generally did not drink this solution except when the tail was pinched. Mean consumption without tail pinch was 0.4 ml for 24 rats.

The volume of milk consumed by each rat was determined and analyzed by two techniques. A linear regression of the dose response curve was used to determine the dose of compound which reduced mean milk consumption by 50% (ED_{50}).

The following drugs and doses were administered: Anorexigens – d-amphetamine 0.3, 1, 3 and 10 mg/kg; chlorphentermine 0.3, 1, 3, 10 and 30 mg/kg; diethylpropion 1, 2, 3 and 10 mg/kg; fenfluramine 0.3, 1, 3 and 10 mg/kg and methamphetamine 0.5, 1 and 2 mg/kg. Depressants – chlordiazepoxide 1, 2 and 4 mg/kg; chlorpromazine 0.1, 0.3, 0.6, 10 and 30 mg/kg; diazepam 0.5, 1, 2, 4 and 8 mg/kg and haloperidol 0.1, 0.3 and 1 mg/kg. Analgesics – morphine 1, 2 and 3 mg/kg and aspirin 10, 30 and 100 mg/kg.

Drugs were dissolved in saline with the addition of a small amount of 1 N HCl as necessary for solution. All solutions were administered IP in a volume of 1 ml/kg except morphine which was administered SC. The times of administration are listed in the tables.

RESULTS

Rats, under the conditions described, consumed 4.5 ± 0.2 ml (mean ± SEM for 86 rats) of this diluted sweetened condensed milk during the 15 min session.

TABLE 1

DOSES OF DRUGS REDUCING STRESS-INDUCED MILK CONSUMPTION TO 50% OF CONTROL VALUES

Compound	Pretreatment min	ED_{50} for anorexigenic activity mg/kg (95% confidence limits)
Anorexigens		
d-amphetamine	15	0.4 (0–1.1)
chlorphentermine	15	2.5 (0.6–9.8)
diethylpropion	15	3.4 (1.8–7.0)
fenfluramine	30	0.9 (0.3–2.1)
methamphetamine	15	1.1 (0.8–1.7)
Analgesic		
morphine	30	1.4 (0.3–2.6)
Depressants		
chlorpromazine	15	9.2 (5.1–19.1)
haloperidol	60	0.3 (0–1.1)

TABLE 2

DOSE OF BENZODIAZEPINES INCREASING STRESS-INDUCED MILK DRINKING TO 133% OF CONTROLS

Compound	Pretreatment min	Dose increasing milk drinking to 133% of controls mg/kg (95% confidence limits)
Chlordiazepoxide	15	1.9 (0.3–11.0)
Diazepam	15	0.8 (0.1–2.8)

Five anorexigens were administered in order to determine their efficacy in antagonizing this milk drinking behavior. All of these agents significantly reduced the milk drinking (Tables 1 and 2).

Morphine was capable of antagonizing the tail-pinch-induced milk drinking; however the dose necessary had marked behavioral effects and these animals were somewhat catatonic. Aspirin, at doses up to 100 mg/kg failed to antagonize this syndrome.

Haloperidol and chlorpromazine were active in preventing this milk drinking syndrome (Table 1), while chlordiazepoxide and diazepam, anxiolytic benzodiazepines, significantly elevated the milk drinking (Table 2).

DISCUSSION

Current methodology for detecting potential anorexigenic agents often lacks relevance to the clinical situation. The use of hypothalamic lesions, starved animals, intracerebrally administered appetite stimulants, and similar methods may not be relevant for detecting agents which reduce human hyperphagia. Clinicians, faced with obese patients, are not faced with starved or lesioned animals. It has been postulated that there was a difference between appetite and hunger [5]. Although this methodology failed to develop a different class of anorexigenic agents, it did recognize the difference between the subject of many pharmacological models, and the hyperphagic patient. The stress-induced hyperphagia model may be a step in this direction [1,4]. This model utilizes rats which had not been food deprived prior to testing. The fact that the rats were totally naive to the situation thereby eliminates problems of learning or prior drug administration.

The necessity for presenting a hand-held drinking burette during the tail-pinch in order to obtain drinking has been clearly described [4]. Since it was difficult to evaluate a reasonable number of rats utilizing hand-held burettes, the use of the cylindrical restraining tube was initiated. In order to somewhat standardize the degree of tail-pinch, the clothespins were selected for their ability to produce an equal amount (although uncalibrated) of pressure. This was accomplished by applying each clothespin to a sealed section of tygon tubing connected to a pressure transducer and a polygraph. Careful positioning of the clothespin produced reproducible measurements. No attempt was made to calibrate this force.

Clinically useful anorexigens, evaluated by this procedure, could be detected. In addition, the narcotic analgesic morphine was also active; however the doses necessary to reduce milk drinking, were behavioral depressive. Aspirin, a nonnarcotic analgesic, did not display any activity in this assay at doses of up to 100 mg/kg, IP.

This tail-pinch-induced hyperphagia has been reported to be dependent upon dopaminergic mechanisms [1,2]. In their work, agents antagonizing dopamine were capable of preventing this hyperphagia. Our results with haloperidol and chlorpromazine confirmed that these compounds reduced the milk consumption. It is a curious paradox that amphetamine and methamphetamine, agents which are known to stimulate dopaminergic receptors, also antagonize this stress-induced hyperphagia. Consequently, the specificity of dopaminergic antagonism as an inhibitor of stress-induced hyperphagia must be questioned.

It is interesting to note that the benzodiazepines have been described to enhance the drinking of sweetened condensed milk, even though this food is novel, and usually rejected by rats. A screening technique for benzodiazepine-like activity based on this enhanced consumption of sweetened condensed milk has been described [3]. In the tests reported herein, an enhancement of the stress-induced hyperphagia was also observed following benzodiazepine administration. This appears to be paradoxical inasmuch as these agents are clinically used to relieve anxiety and

presumably reduce stress. Obviously, they do not reduce this type of stress.

The method as described is a useful initial test for anorexigenic compounds. Agents which are active in this test, however, must be evaluated for analgesia and other depressant behavioral effects. This method will also detect benzodiazepine-like activity as an increase in milk consumption as described both herein and elsewhere [3].

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